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# Optical Control of L-Type Ca<sup>2+</sup> Channels Using a Diltiazem Photoswitch

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**L-type  $\text{Ca}^{2+}$  channels (LTCCs) play a crucial role in excitation-contraction coupling and the release of hormones from secretory cells. They are targets of numerous antihypertensive and antiarrhythmic drugs such as diltiazem. Here, we present a photoswitchable derivative of diltiazem, FHU-779, which can be used to reversibly block endogenous LTCCs by light. FHU-779 is almost as potent as diltiazem itself, and can be used to place pancreatic  $\beta$  cell function and cardiac activity under optical control.**

L-type  $\text{Ca}^{2+}$  channels (LTCCs,  $\text{Ca}_v1$ ) serve fundamental physiological roles in excitable tissues<sup>1</sup>. Activated by membrane depolarization, LTCCs permit  $\text{Ca}^{2+}$  influx into cells and are key players in voltage-dependent signal transduction<sup>1,2</sup>. For instance, LTCCs are crucial for pacemaking, electrical conduction, and excitation-contraction coupling in the heart<sup>1</sup>. In pancreatic  $\beta$  cells, LTCCs are essential for excitation-secretion coupling affording insulin release<sup>3</sup>. To study the role of LTCCs in more detail,  $\text{Ca}^{2+}$  imaging techniques have been applied for monitoring the cytosolic  $\text{Ca}^{2+}$  concentration in living cells with high resolution<sup>4,5</sup>. However, tools to control  $\text{Ca}^{2+}$  influx through endogenous LTCCs with comparable temporal and spatial resolution have been lacking so far. We have addressed this experimental need by creating a photoswitch that enables control of LTCCs using light.

Based on the structure of the benzothiazepine-like  $\text{Ca}^{2+}$  channel blocker diltiazem, we synthesized the doubly-charged, hydrophilic photoswitch FHU-779 by installing a photoresponsive azobenzene moiety (**Fig. 1a,b**; for synthesis see **Supplementary Note**). Under blue (470 nm) illumination or in the dark, the compound rests in its thermally more stable *trans*-state. Upon UV-A irradiation (385 nm), the compound is reversibly converted into its energetically elevated *cis*-state, which has a bent conformation. We characterized the spectral properties of *cis*- and *trans*-FHU-779 by UV-Vis spectroscopy (**Fig. 1c**). Thermal relaxation from *cis*- to *trans*-FHU-779 detected at 360 nm followed a mono-exponential time course with a time constant of  $\tau = 4081 \pm 71$  min (**Supplementary Fig. 1**). To determine whether FHU-779 was able to afford optical control of LTCC function, we performed whole-cell patch clamp recordings using HEK293T cells heterologously expressing  $\text{Ca}_v1$  channels. Current-voltage (I-V) relationships for  $\text{Ca}_v1.2$  and  $\text{Ca}_v1.3$  in the

presence of *cis*- and *trans*-FHU-779 are shown in **Fig. 1d** and **Supplementary Fig. 2**, respectively ( $n = 3$  cells each). In its *trans*-state, FHU-779 strongly reduced inward currents conducted by both channel subtypes. The block of  $\text{Ca}_v1$  channels was reversible when FHU-779 was repeatedly photoswitched between its *trans*- and *cis*-states (**Fig. 1e,f**; **Supplementary Fig. 2**). Quantification of block induced by 25  $\mu\text{M}$  externally applied FHU-779 was impeded by LTCC current rundown<sup>6</sup>, yielding photoswitching efficacies of  $46.5 \pm 7.6\%$  and  $46.4 \pm 5.4\%$  for  $\text{Ca}_v1.2$  ( $n = 10$  cells) and  $\text{Ca}_v1.3$  channels ( $n = 9$  cells), respectively (**Fig. 1g**). In contrast, FHU-779-mediated photoswitching of voltage-gated potassium ( $\text{K}_v$ ) channels, which are reportedly blocked by high dose diltiazem<sup>7</sup>, was far less efficient with only  $6.2 \pm 2.7\%$  ( $n = 6$  cells) and  $5.9 \pm 3.0\%$  ( $n = 6$  cells) photoswitching detected for heterologously expressed Shaker-IR or  $\text{K}_v$  channels, respectively. Also, photoswitching quantification against voltage-gated sodium channel  $\text{Na}_v1.5$  by FHU-779 showed only  $5.4 \pm 0.9\%$  change ( $n = 10$  cells). These data demonstrate that FHU-779 endows light sensitivity to the two major LTCC subtypes,  $\text{Ca}_v1.2$  and  $\text{Ca}_v1.3$ , but has negligible effects on  $\text{K}_v$  and  $\text{Na}_v$  channels. In an attempt to determine the access route of FHU-779 into LTCCs, the drug was applied intracellularly via the patch pipette. Whole-cell dialysis of 100  $\mu\text{M}$  FHU-779 resulted in negligible photoswitching of both  $\text{Ca}_v1.2$  ( $8.6 \pm 6.0\%$ ;  $n = 4$  cells) and  $\text{Ca}_v1.3$  currents ( $2.0 \pm 4.6\%$ ;  $n = 7$  cells, **Fig. 1g**). This result suggests that FHU-779 enters LTCCs from the extracellular side rather than from the cytosol<sup>8</sup>.

To more precisely quantify FHU-779 photoswitching, we loaded HEK293T cells heterologously expressing a  $\text{Ca}_v1.2$  mutant deficient for channel inactivation ( $\text{Ca}_v1.2\Delta1671$ )<sup>9</sup> with the fluorescent  $\text{Ca}^{2+}$  indicator Fura-2 and performed ratiometric  $\text{Ca}^{2+}$  imaging<sup>10</sup>. LTCC channels were activated by membrane depolarization with high extracellular  $[\text{K}^+]$ , and the consecutive increase in intracellular  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_i$ ) was recorded (**Fig. 2a**; **Supplementary Fig. 3**; **Supplementary Movies 1-3** and online methods). Subsequent wash-in of FHU-779 decreased cytosolic  $\text{Ca}^{2+}$  levels in the dark, whereas changing illumination wavelengths between 480 nm and 380 nm allowed for reliable optical control of  $\text{Ca}^{2+}$  influx into cells.

The  $\text{Ca}^{2+}$  imaging based quantification of  $\text{Ca}_v1.2$  block by diltiazem yielded a dose-response relationship with an  $\text{IC}_{50}$  of  $2.7 \mu\text{M} \pm 1.0\%$  (**Supplementary Fig. 3**), which is in accordance with the literature<sup>11,12</sup> and in the same range as *trans*-FHU-779 showing an  $\text{IC}_{50}$  of  $8.3 \mu\text{M} \pm 0.6\%$  (**Fig. 2b**). The difference between both dose-response relationships

of *trans*-FHU-779 and *cis*-FHU-779 data represents quantitative photoswitching amplitudes at a given concentration.

To rationalize these results, we constructed an open-state Ca<sub>v</sub>1.2 model and docked FHU-779 using a previously elaborated approach<sup>13</sup> with the open Na<sub>v</sub>Ab channel X-ray structure as a template<sup>14</sup> (**Fig. 2c,d**; **Supplementary Fig. 4,5**; **Supplementary Table 1,2**; online methods). The model accommodated *trans*-FHU-779 with the tricyclic moiety and the long chain as predicted for benziazem<sup>15</sup>, which shares a similar chemical structure as FHU-779. The benzothiazepine moiety was bound in the repeat III/IV fenestration, the protonated nitrogen resided near the selectivity filter, and the long photoswitchable moiety extended along the inner pore (**Fig. 2c**). *cis*-FHU-779 was also accommodated in the inner cavity, but the folded conformation was too bulky to access this site either through the open activation gate or a fenestration (**Fig. 2d**).

To further explore the application spectrum of FHU-779 in primary cells and tissue endogenously expressing LTCCs, we used pancreatic islets and monitored [Ca<sup>2+</sup>]<sub>i</sub> in β cells by Fluo-8 fluorescence intensity imaging (**Fig. 3a**). When triggered by LTCC-mediated Ca<sup>2+</sup> influx, pancreatic β cells secrete insulin to regulate blood glucose levels<sup>16</sup>. First, the sulfonylurea and ATP-sensitive K<sup>+</sup> channel (K<sub>ATP</sub>) blocker tolbutamide was applied to depolarize β cells and fully activate LTCCs<sup>17</sup>. This led to Ca<sup>2+</sup> influx that was strongly reduced by *trans*-FHU-779 when cells were illuminated at 470 nm (**Fig. 3a**). This effect was reversed by illumination at 395 nm, yielding 52.2 ± 2.7% photoswitching (n = 9 islets, see online methods). Controls were performed relative to the magnitude of tolbutamide alone, and with diltiazem- and tolbutamide-treated islets (n = 9, **Supplementary Fig. 6**). Prior application of a saturating concentration of diltiazem precluded photoswitching by FHU-779 (**Supplementary Fig. 6**). Whole-cell patch clamp experiments in dissociated pancreatic β cells displayed reduced inward currents through LTCCs at 470 nm as compared to 370 nm light in the presence of FHU-779 (**Fig. 3b**), reinforcing our imaging results. In the absence of FHU-779, corresponding I-V relationships did not differ in current amplitude in response to illumination (**Supplementary Fig. 6**). Insulin secretion was determined in islets under control conditions (tolbutamide), or following application of diltiazem and *cis*- or *trans*-FHU-779 (**Fig. 3c**). As expected, only diltiazem and *trans*-FHU-779 application were able to significantly reduce insulin secretion. To demonstrate the spatiotemporal control of LTCCs, we reversibly photoactivated FHU-779 in a single

from a doublet or triplet islets of Langerhans (**Supplementary Fig. 7**), as well as in single beta cells residing within the intact microorgan (**Fig. 3d-f**), while performing  $\text{Ca}^{2+}$  imaging. As such, FHU-779 enables robust and precise spatiotemporal optical control of endogenous LTCC function,  $\text{Ca}^{2+}$  fluxes and insulin secretion in pancreatic  $\beta$  cells.

FHU-779 was also applicable to explanted hearts perfused in the Langendorff configuration. Besides coupling excitation and contraction in cardiomyocytes, LTCCs are important for sinoatrial pacemaking in the right atrium and atrioventricular conduction<sup>1</sup>. FHU-779 was perfused into coronary arteries and the right atrium was illuminated. In line with the LTCC-block by *trans*-FHU-779, illumination with 480 nm reduced heart rate, which was quickly reversed by 380 nm illumination (**Fig. 3g**). Photoswitching significantly affected the heart rate by  $5.8 \pm 1.8\%$  ( $n = 4$  hearts) in comparison to control experiments, which were performed prior to FHU-779 application but with the same illumination protocol (**Fig. 3h**). We also screened for the optimal irradiation wavelength, and found that 360 nm illumination achieved the largest increase in heart rate while 400 nm light was still effective (**Supplementary Fig. 8**). Furthermore, we analyzed the relationship between cycle length for switching between 480 nm and 380 nm illumination and heart rate response and found a maximal effect at 30 s of illumination (**Supplementary Fig. 8**). These experiments were further substantiated by whole-cell patch-clamp recordings of LTCC currents from adult cardiomyocytes and revealed photoswitching of  $20.5 \pm 8.8\%$  ( $n = 12$  cells) (**Supplementary Fig. 8**). To demonstrate spatial control of cardiomyocyte function, Cor4U cardiomyocytes were plated on microelectrode arrays (MEA) and treated with FHU-779. The duration of extracellular field potentials (FPs), which is reflecting the LTCC-dependent plateau phase of the cardiac action potential<sup>18</sup> was measured from individual electrodes, and only part of the MEA was illuminated with either 385 nm or 480 nm light. The illuminated electrodes showed a FP prolongation upon UV-light and FP shortening using 480 nm indicating activation and block of LTCCs, respectively (**Fig. 3i**). This effect could be changed repetitively and was not observed on non-illuminated electrodes of the same MEA (**Supplementary Fig. 9**).

In conclusion, we present a tool to reversibly control one of the main gates of  $\text{Ca}^{2+}$  influx into excitable cells, the LTCCs, at spatiotemporal precision of light. Derived from the clinically used LTCC blocker diltiazem, our molecule is applicable in dissociated primary

cells and *ex vivo* organ preparations. In contrast to genetically encoded optogenetic tools, such as ChR2<sup>19</sup>, OptoSTIM1<sup>20</sup>, or Opto-CRACK<sup>20</sup>, FHU-779 bestows light sensitivity to *endogenous* LTCCs. Compared to light-flash photolysis of nifedipine for optical control of LTCC activity<sup>21,22</sup>, FHU-799 extends experimental options by adding a high temporal reversibility. Thus, biological processes involving LTCC-related Ca<sup>2+</sup>oscillations may now be accessed; also, localized modulation of LTCC-induced cardiac arrhythmia can be envisaged. Since FHU-779 serves as new lead structure, further chemical modifications will multiply specific LTCC tools and thus biological applications.

### **Author contribution:**

The project was conceived by T.F., N.K. and D.T.. Patch clamp characterization of FHU-779 was carried out by T.F., J.F., D.M., T.B. and J.G.D.. Ratiometric Ca<sup>2+</sup> imaging in HEK293T cells was performed by T.F. and M.S.. Ca<sup>2+</sup> imaging of pancreatic islets was carried out by N.H.F.F and D.J.H.. Heart rate modulation on Langendorff-perfused hearts was performed by T.B., T.F. and P.S. and FP experiments by D.M. and P.S.. Molecular modeling was performed by D.B.T. and B.S.Z.. Synthesis of FHU-779 was carried out by F.M.E.H. and N.H.. T.F., D.T. and N.K. wrote the manuscript with contributions from all authors.

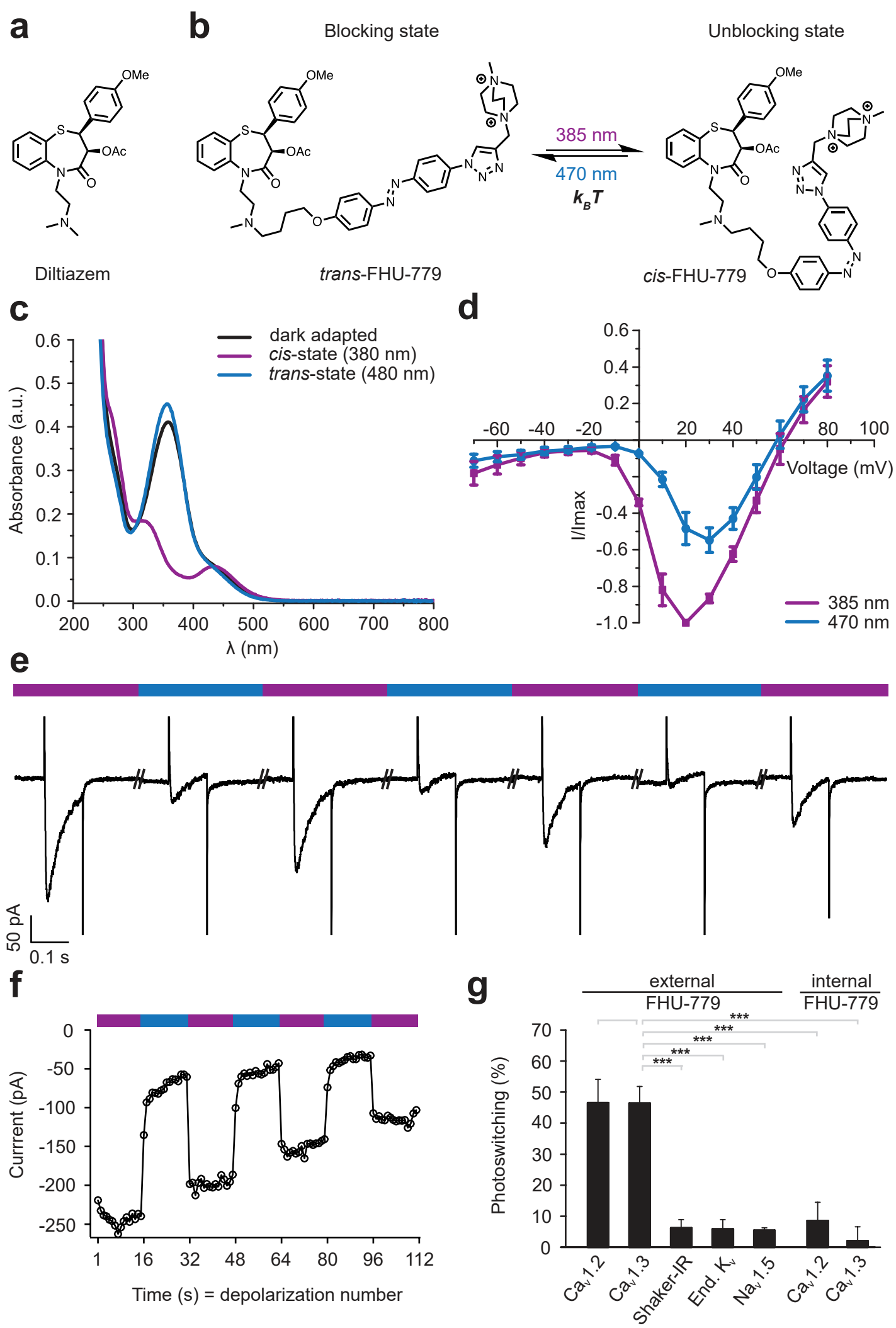
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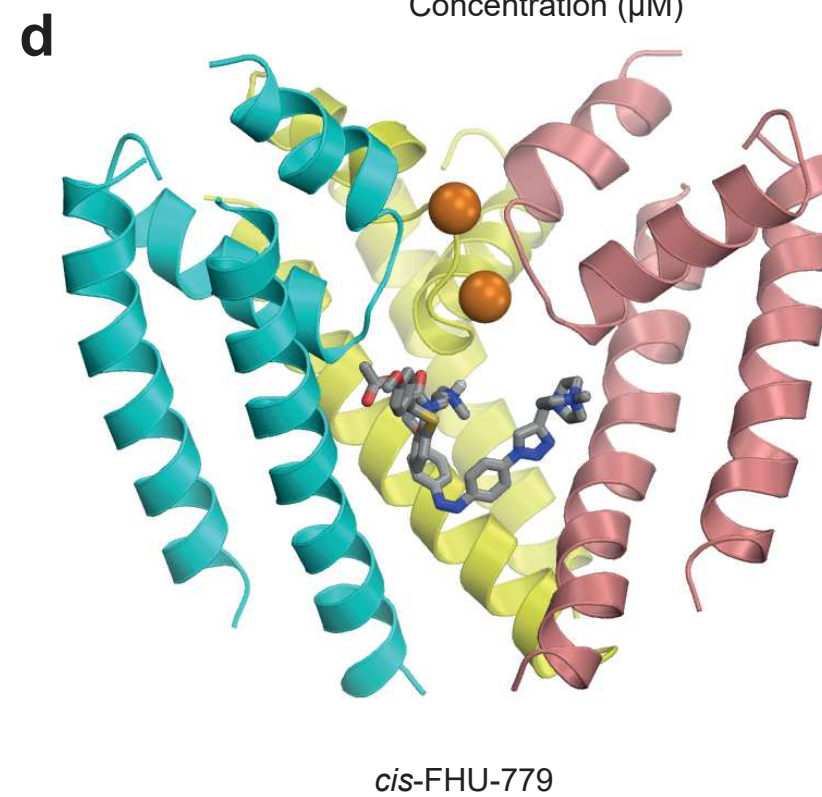
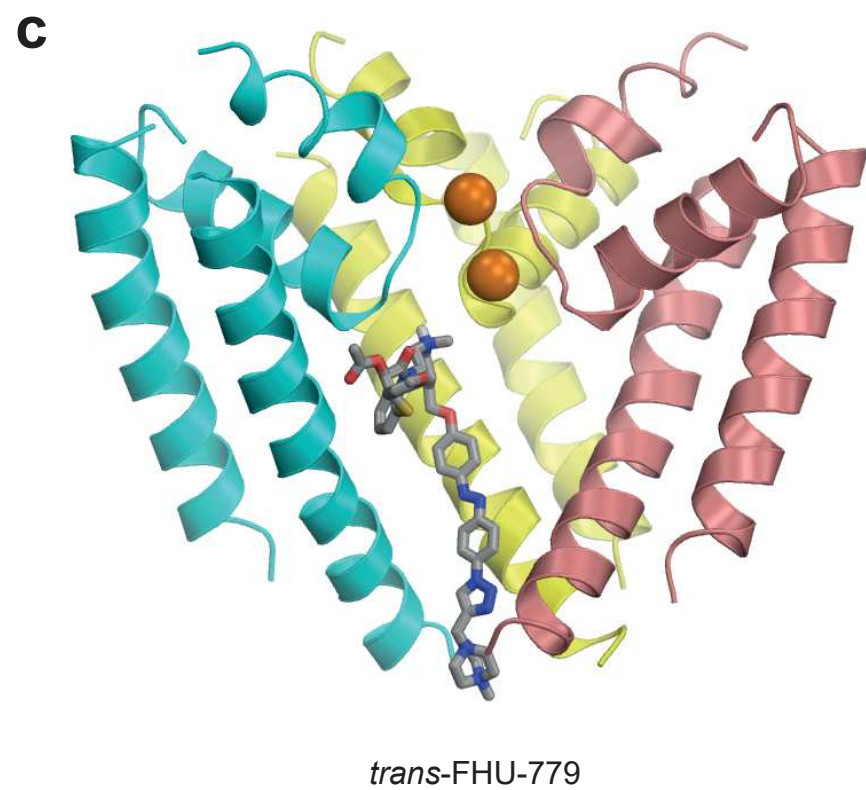
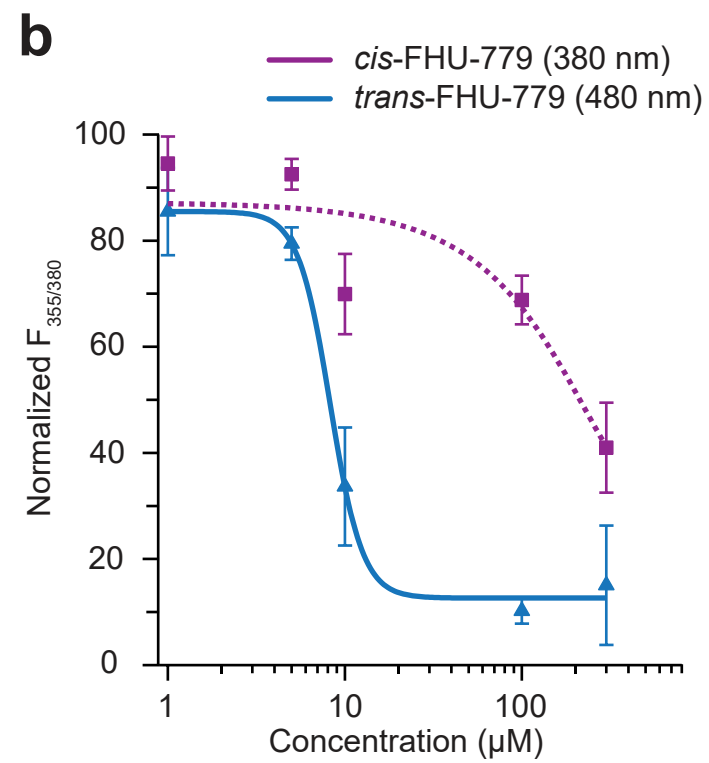
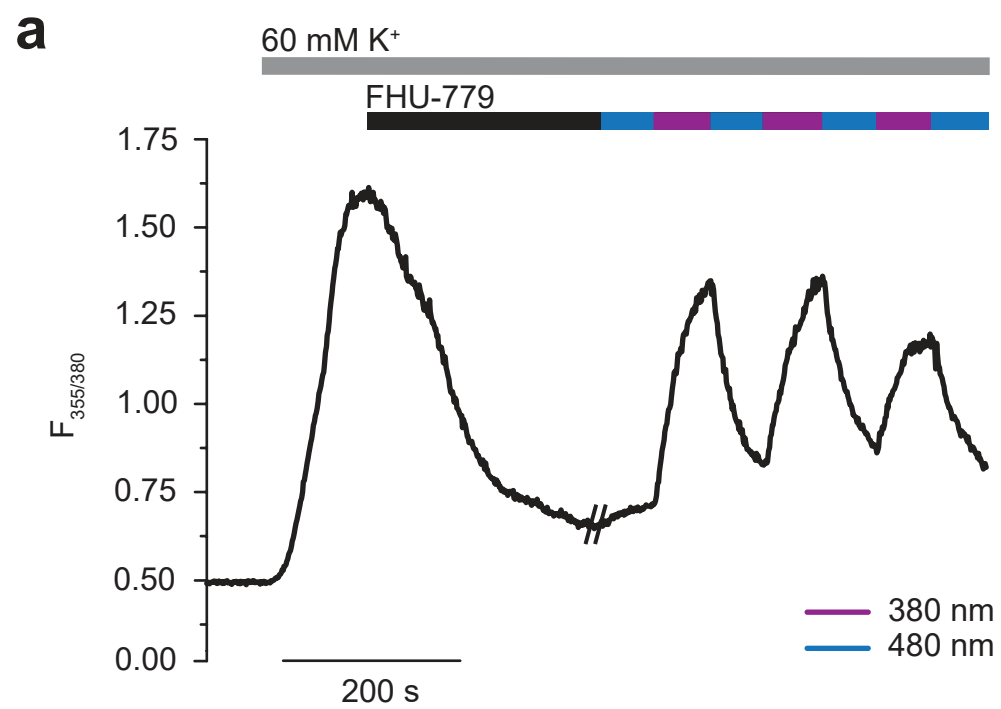
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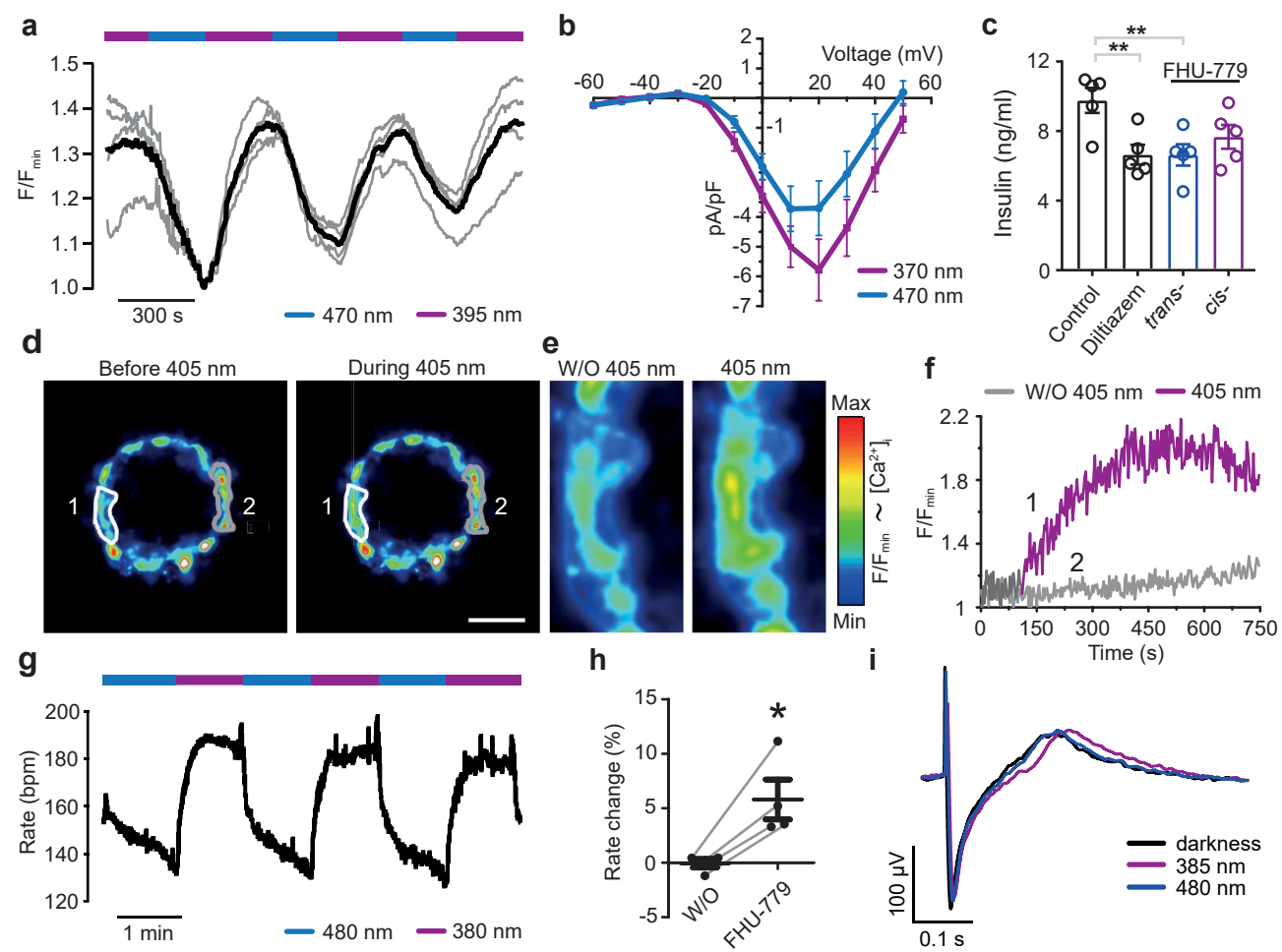
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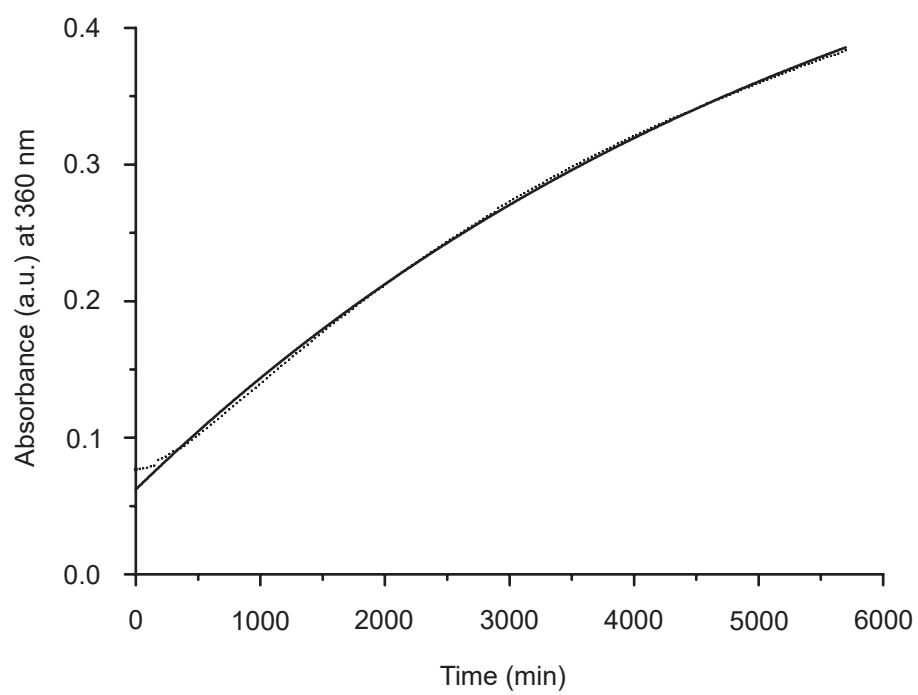


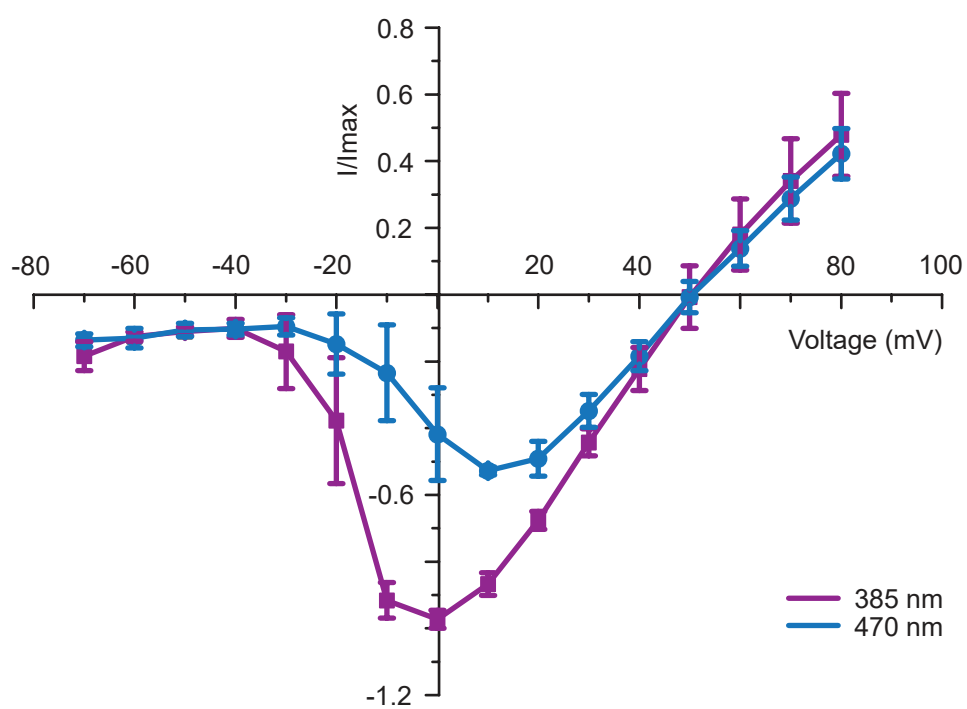
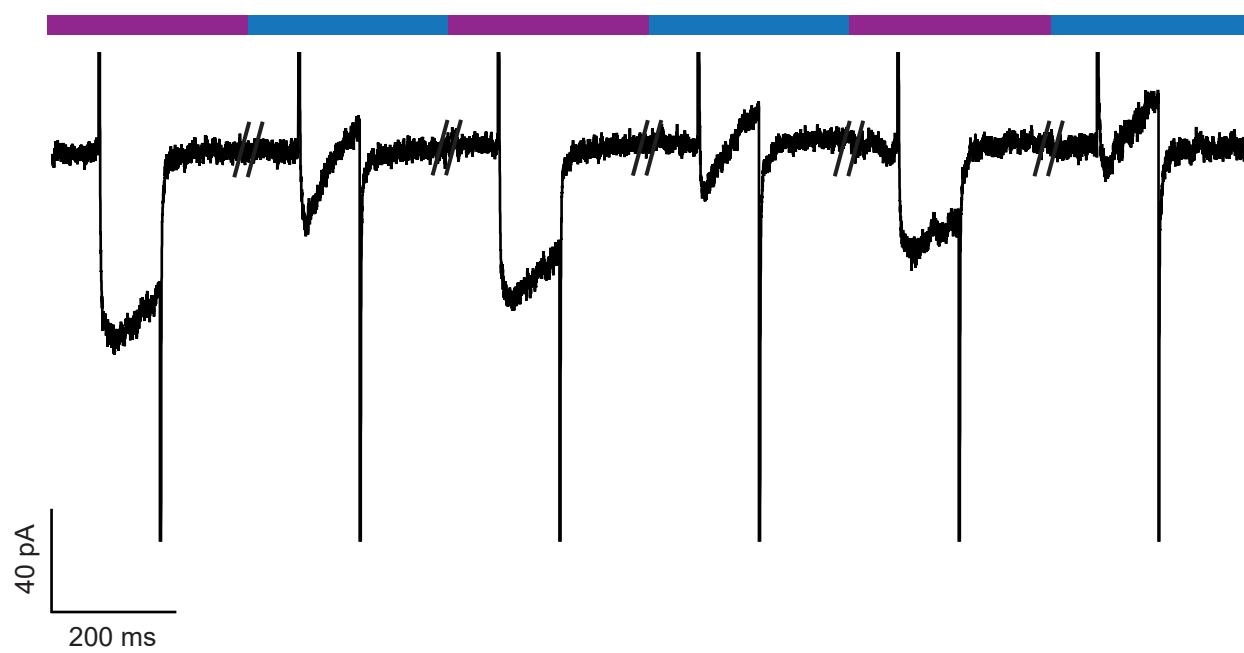
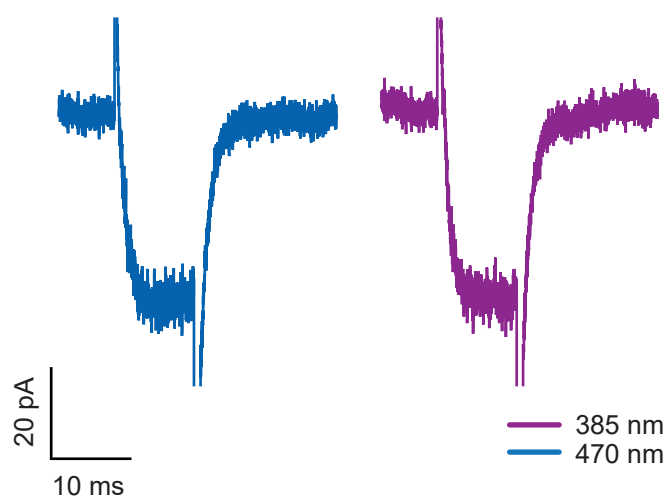
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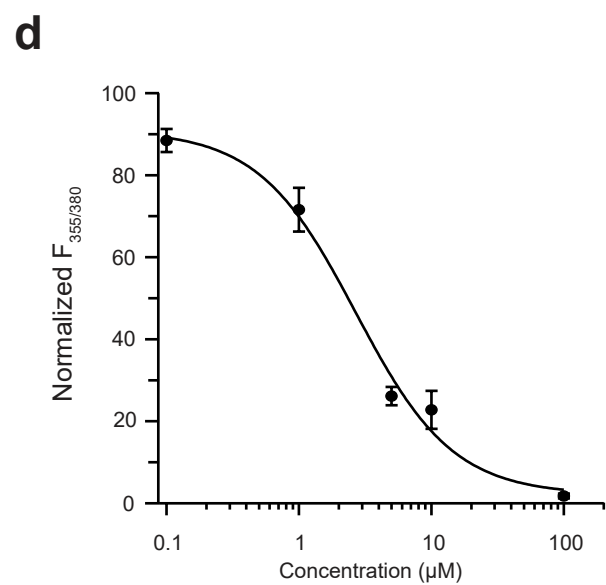
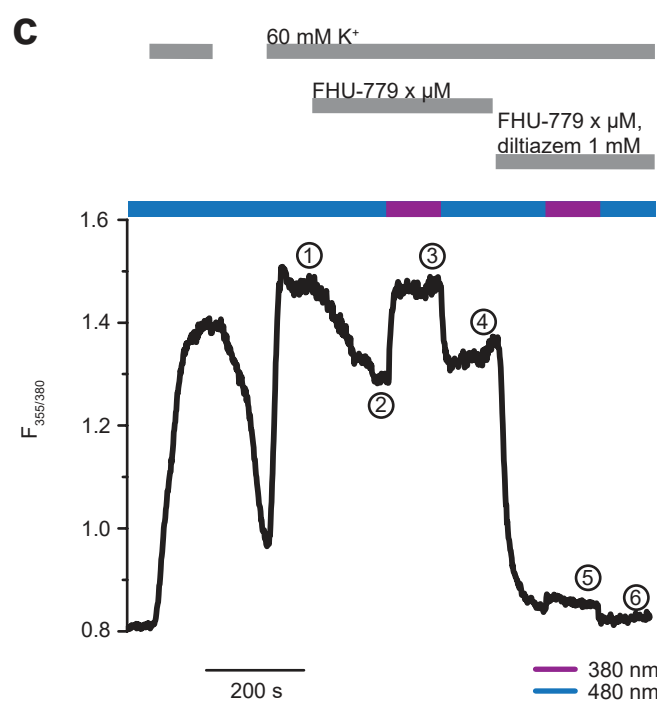
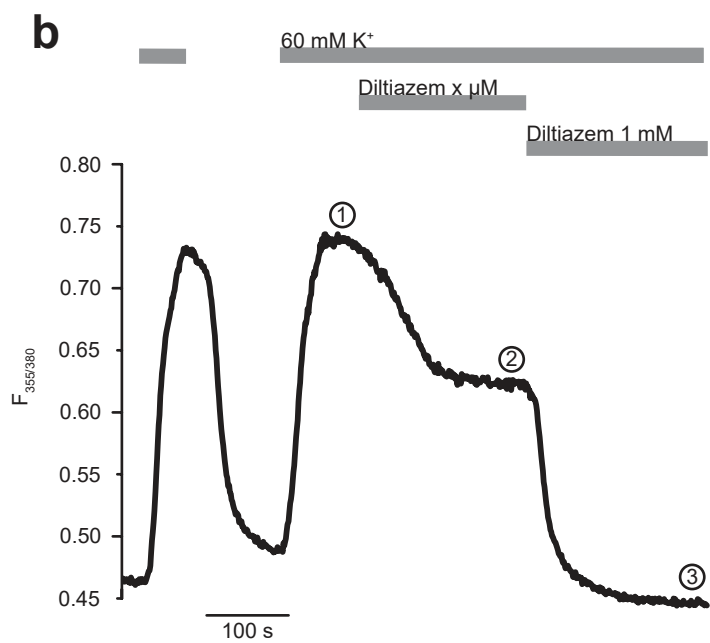
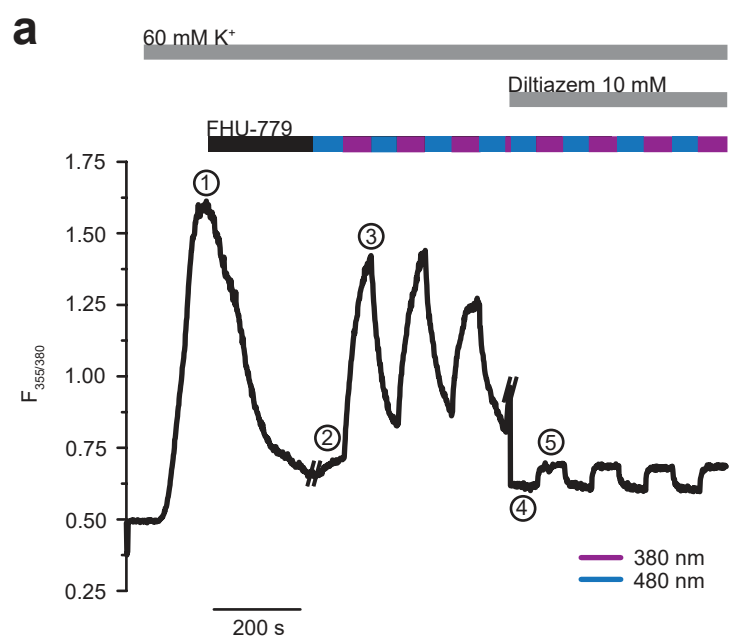




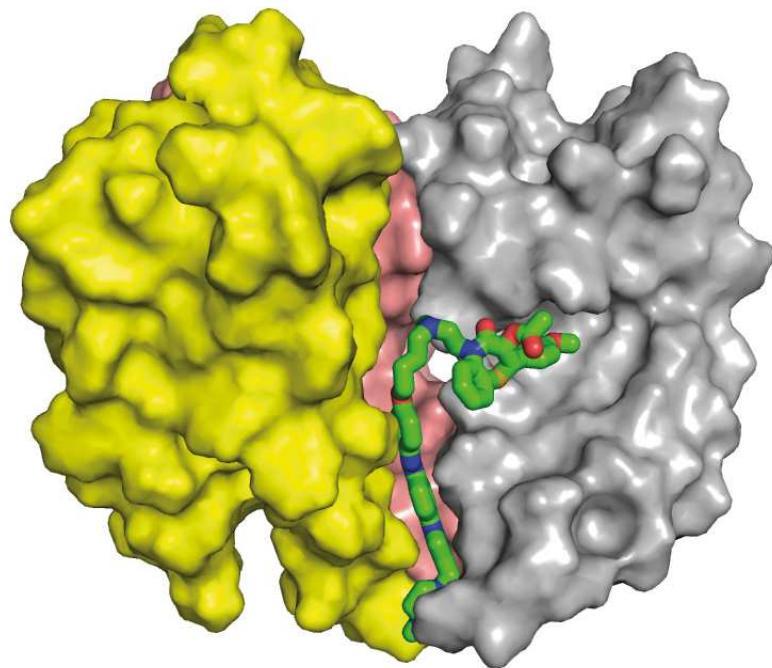




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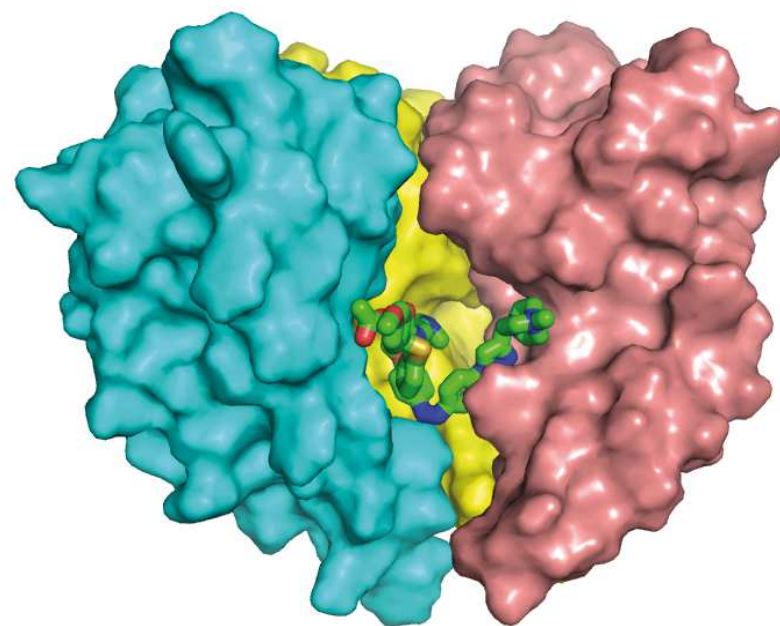


**a**



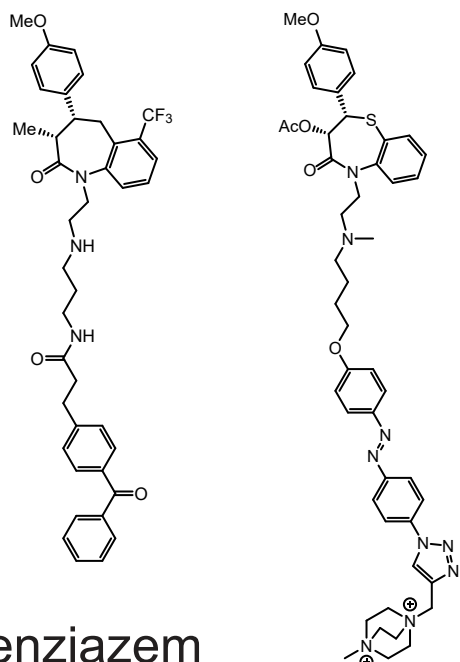
*trans*-FHU-779

**b**

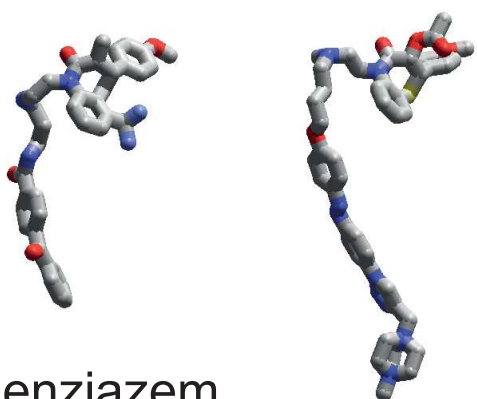
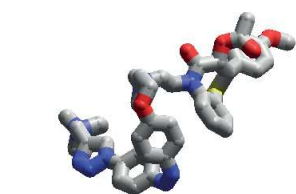
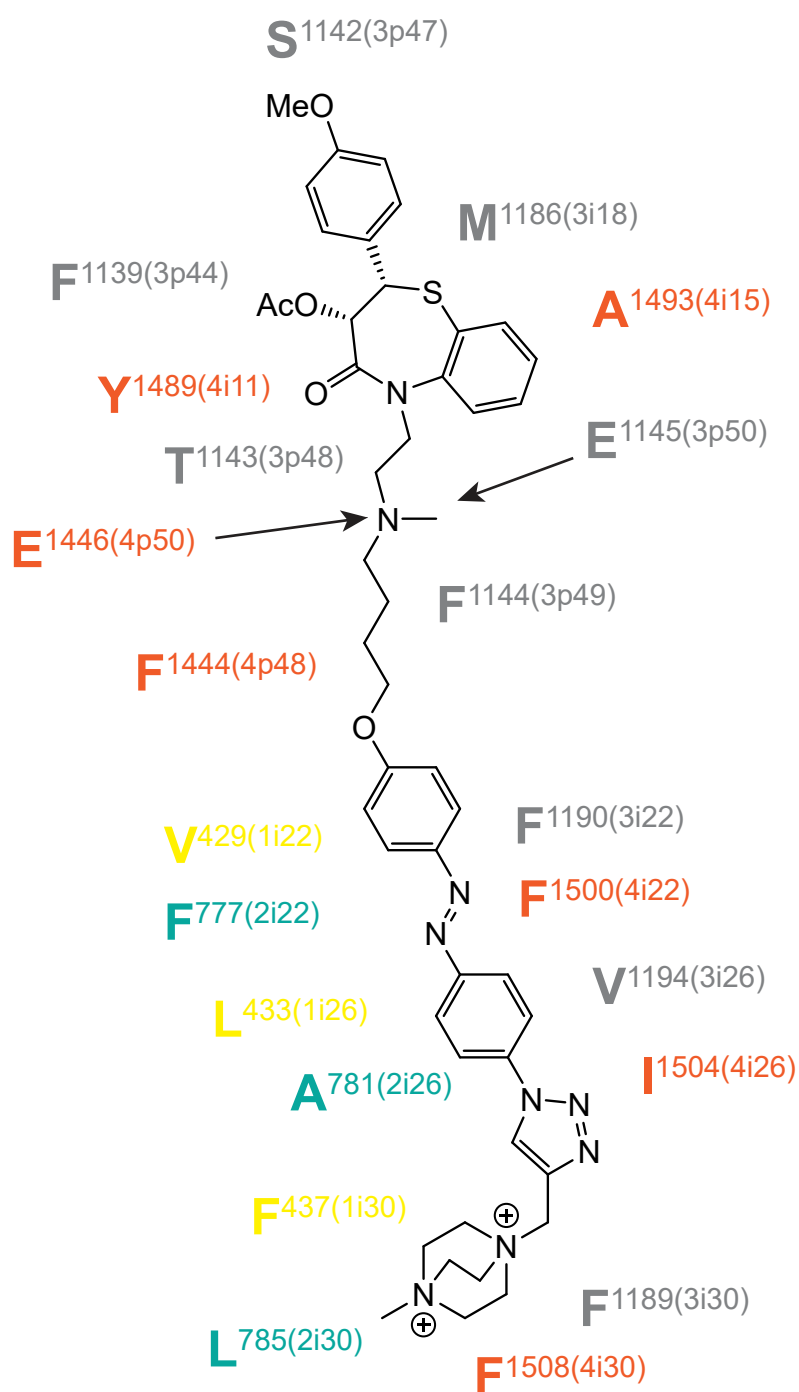


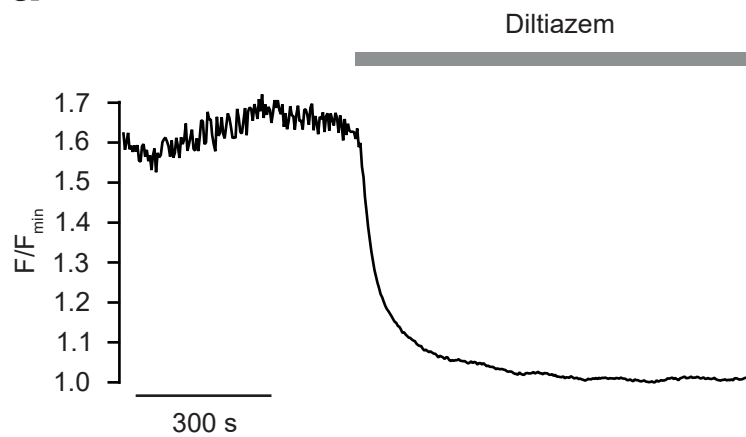
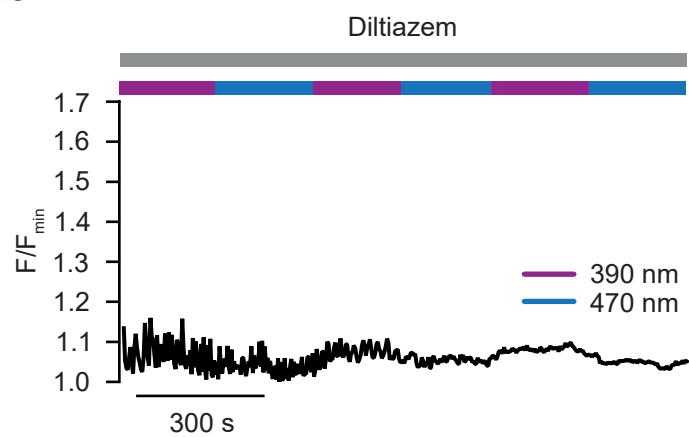
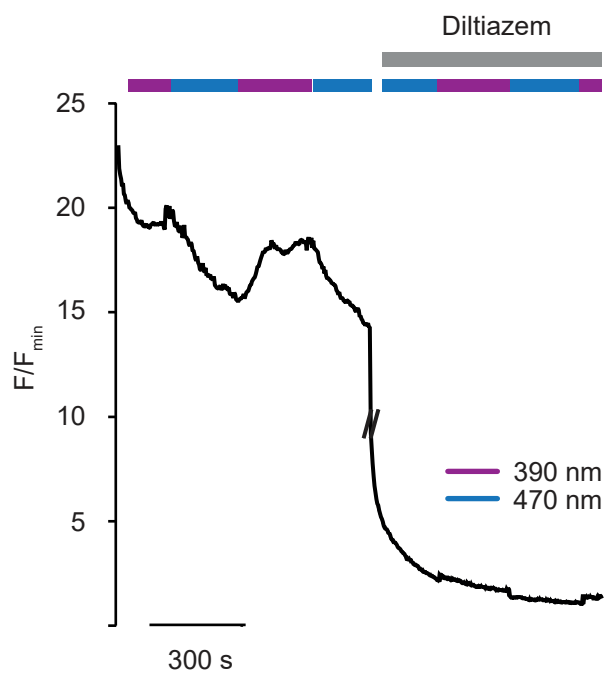
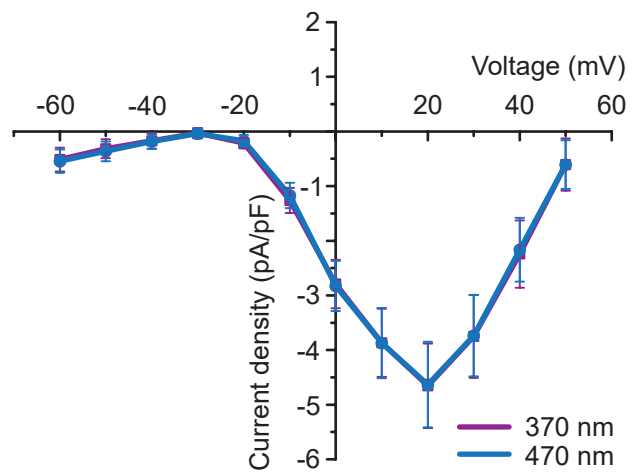
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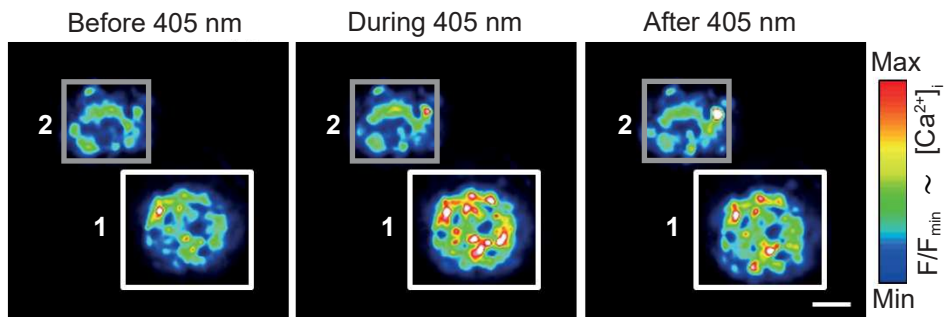
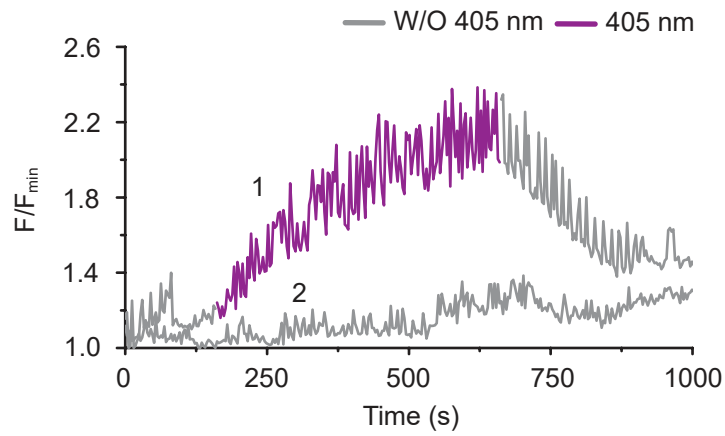
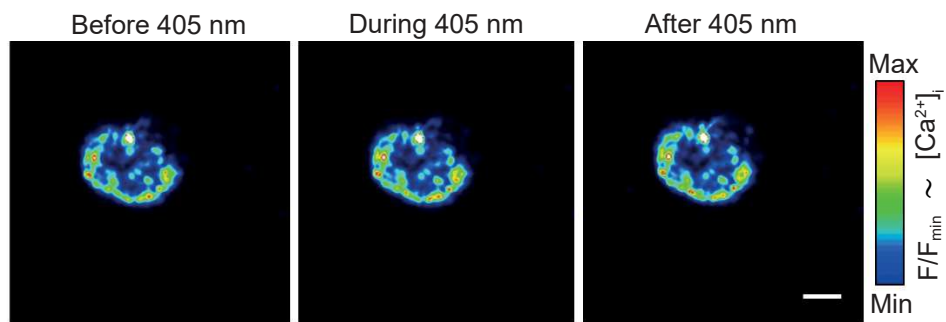
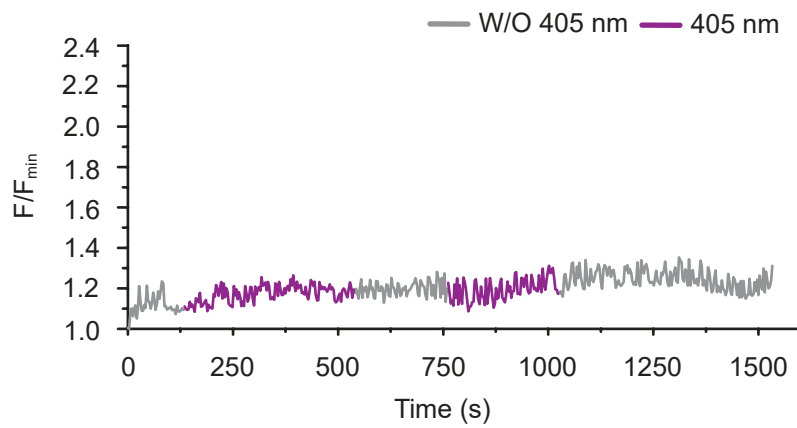


**a**Benziazem  
derivative

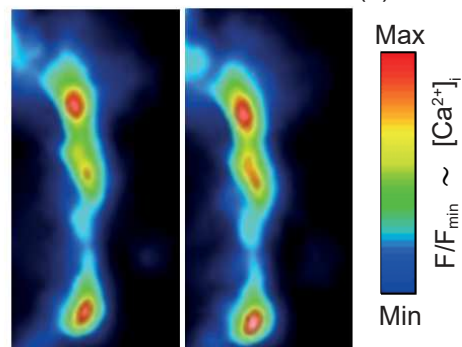
FHU-779

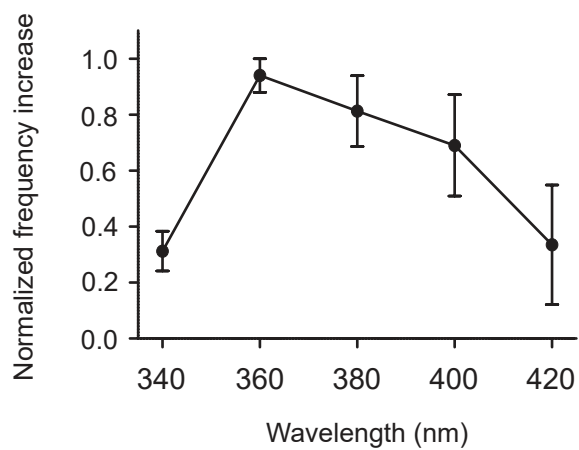
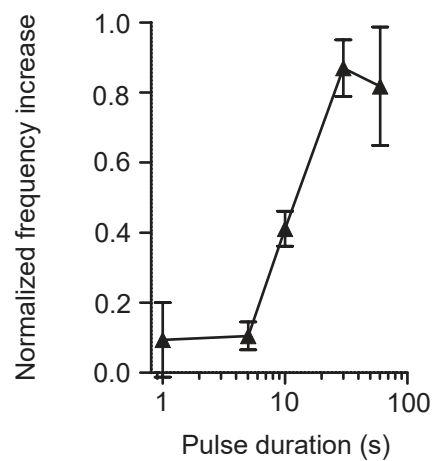
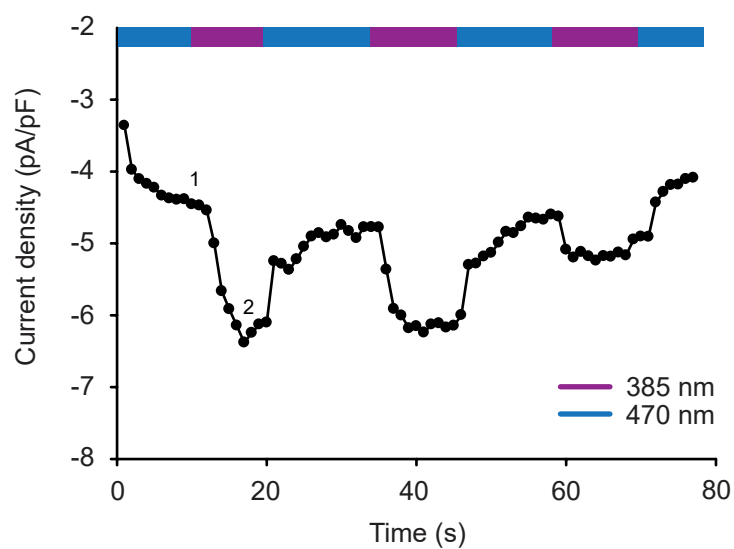
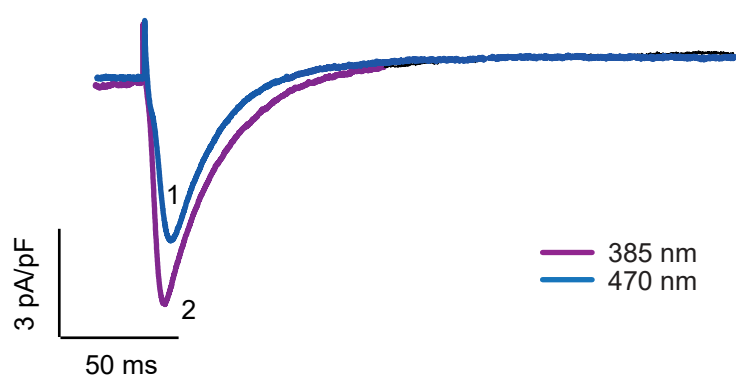
**b**Benziazem  
derivative*trans*-FHU-779*cis*-FHU-779**c**

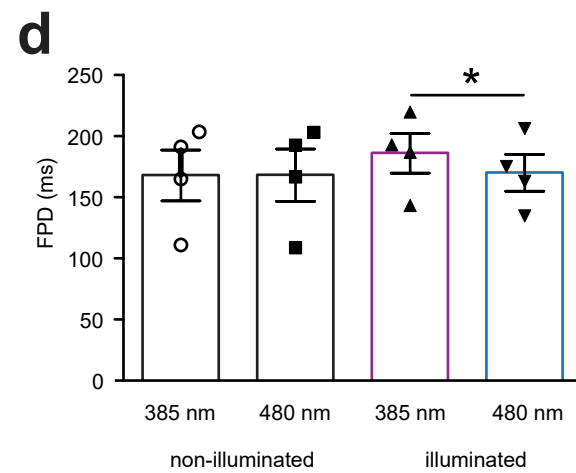
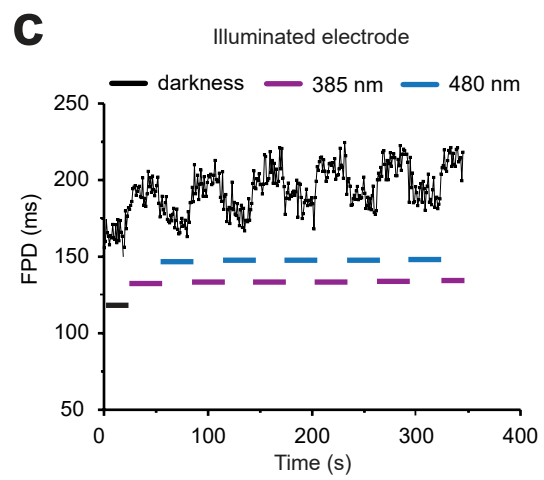
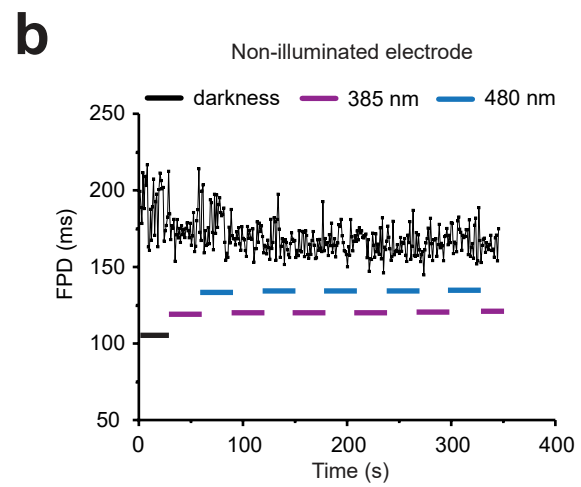
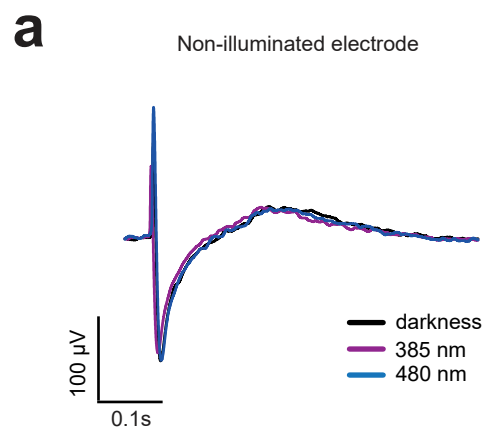
**a****b****c****d**

**a****b****c****d****e**

W/O 405 nm    405 nm in ROI (1)



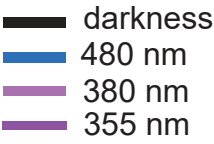
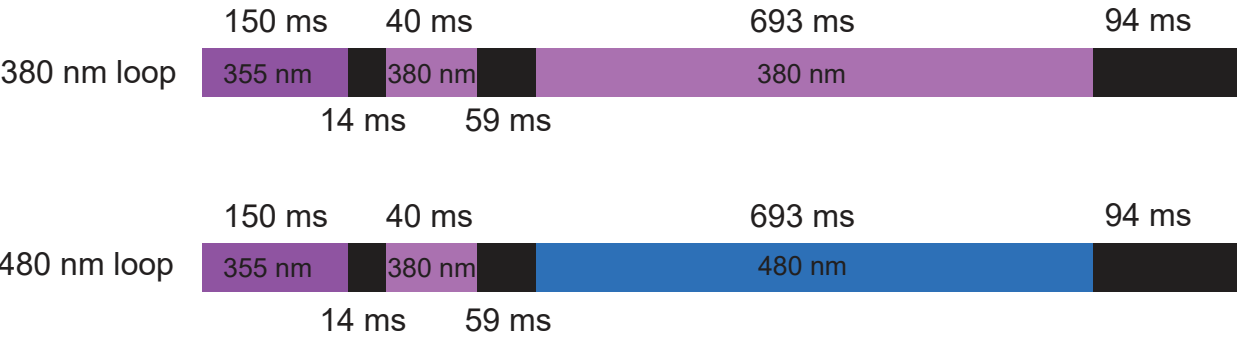
**a****b****c****d**



**a**



**b**



**Supplementary Table 1.** Sequence alignment and benzothiazepine-sensing residues

Channel	Segment	# <sup>a</sup>	Sequence <sup>b,c</sup>		
			p40	p50	
Na <sub>v</sub> Ab	P	1159	WFGLGESF	YTLFQVMTLE	SWSMGIV
Ca <sub>v</sub> 1.2	IP	375	FDNFAM	LTVFQCITME	GWTDVLY
	IIP	719	FDNFQSL	LTVFQILTGE	DWNSVMY
	IIIP	1128	FDNVLAAM	MALFTVST <b><u>FE</u></b>	GWPELLY
	IVP	1429	FQTFPQAV	LLLFRCAT <b><u>E</u></b>	AWQDIML
			i10	i20	i30
Na <sub>v</sub> Ab	S6	1193	YAWVFFIPF	IFVVFFVMIN	LYVAIIVDAMAI
Ca <sub>v</sub> 1.2	IS6	410	LPWVYFVSL	VIFGSFFVLN	LVLGVLSGEFSK
	IIS6	758	LVCIFYFIIL	FICGNYILLN	VFLAIAVDNLAD
	IIIS6	1171	EISIFF <b><u>I</u></b> IY	<b><u>I</u></b> <b><u>I</u></b> <b><u>I</u></b> <b><u>I</u></b> AFF <b><u>M</u></b> MN	<b><u>I</u></b> <b><u>FV</u></b> GFVIVTFQE
	IVS6	1481	FAVFYF <b><u>I</u></b> SF	<b><u>Y</u></b> <b><u>M</u></b> <b><u>L</u></b> <b><u>C</u></b> <b><u>A</u></b> <b><u>F</u></b> <b><u>L</u></b> <b><u>I</u></b> IN	LFVAVIMDNFDY

<sup>a</sup> Residue numbers in the Ca<sub>v</sub>1.2 channel are given according UniProtKB - P15381 (CCAC\_RABIT) and in the Na<sub>v</sub>Ab channel according to X-ray structures (PDB codes 5VB8, 5VB2).

<sup>b</sup> Labels, which are universal for P-loop channels<sup>1</sup>, are shown above respective sequences.

<sup>c</sup> Experimentally determined benzothiazepine-sensing residues<sup>2-4</sup> are bold-typed and underlined.

**Supplementary Table 2.** Interaction energy of *trans*- and *cis*-FHU-779 with the Ca<sub>v</sub>1.2 model partitioned by the channel residues <sup>a</sup>

P-loops	Energy, kcal/mol		S6 <i>i11-i22</i>	Energy, kcal/mol		S6 <i>i23-i30</i>	Energy, kcal/mol	
	<i>trans</i> -	<i>cis</i> -		<i>trans</i> -	<i>cis</i> -		<i>trans</i> -	<i>cis</i> -
<u>F<sup>1139</sup>(3p44)</u>	-0.70	-2.98	<u>I<sup>1179</sup>(3i11)</u>	<u>-1.11</u>	<u>-1.16</u>	<u>L<sup>778</sup>(2i23)</u>	-0.58	
<u>S<sup>1142</sup>(3p47)</u>	-1.97	-1.17	<u>Y<sup>1489</sup>(4i11)</u>	<u>-3.48</u>	<u>-2.20</u>	<u>L<sup>1191</sup>(3i23)</u>	-0.9	
<u>A<sup>1443</sup>(4p47)</u>	-0.68	-2.39						
<u>T<sup>1143</sup>(3p48)</u>	-2.37	-0.73	<u>I<sup>1180</sup>(3i12)</u>		-0.53	<u>L<sup>433</sup>(1i26)</u>	-3.05	
<u>T<sup>1444</sup>(4p48)</u>	-3.11	-2.72	<u>M<sup>1490</sup>(4i12)</u>	<u>-1.19</u>	<u>-1.19</u>	<u>A<sup>781</sup>(2i26)</u>	-1.28	
<u>M<sup>391</sup>(1p49)</u>	-1.03	-0.75				<u>V<sup>1194</sup>(3i26)</u>	-1.08	
<u>F<sup>1144</sup>(3p49)</u>	<u>-2.22</u>	<u>-2.57</u>	<u>I<sup>1182</sup>(3i14)</u>	<u>-1.12</u>	<u>-0.88</u>	<u>I<sup>1504</sup>(4i26)</u>	-1.16	
<u>E<sup>1145</sup>(3p50)</u>	<u>-0.90</u>	<u>-0.70</u>						
<u>E<sup>1446</sup>(4p50)</u>	<u>-0.73</u>	<u>-0.67</u>	<u>A<sup>1493</sup>(4i15)</u>	<u>-3.13</u>	<u>-1.75</u>	<u>F<sup>437</sup>(1i30)</u>	-1.62	
			<u>F<sup>1494</sup>(4i16)</u>	-0.80		<u>L<sup>785</sup>(2i30)</u>	-1.13	
			<u>F<sup>1185</sup>(3i17)</u>	-0.52	-0.72	<u>F<sup>1198</sup>(3i30)</u>	-1.45	
						<u>F<sup>1508</sup>(4i30)</u>	-1.52	
			<u>V<sup>425</sup>(1i18)</u>		-0.75			
			<u>L<sup>773</sup>(2i18)</u>		-2.21			
			<u>M<sup>1186</sup>(3i18)</u>	<u>-2.22</u>	<u>-3.87</u>			
			<u>I<sup>1496</sup>(4i18)</u>	<u>-0.93</u>				
			<u>L<sup>426</sup>(1i19)</u>	-1.21	-1.77			
			<u>L<sup>774</sup>(2i19)</u>		-1.28			
			<u>M<sup>1187</sup>(3i19)</u>	-0.80				
			<u>I<sup>1497</sup>(4i19)</u>	-1.44				
			<u>V<sup>429</sup>(1i22)</u>	-2.04	-1.13			
			<u>F<sup>777</sup>(2i22)</u>	-0.61	-6.40			
			<u>F<sup>1190</sup>(3i22)</u>	<u>-3.65</u>	<u>-3.26</u>			
			<u>F<sup>1500</sup>(4i22)</u>	-3.55	-2.68			
Total	-14.91	-18.26	Total	-28.55	-35.14	Total	-15.90	0.00
<b><i>trans</i>-FHU-779 Total</b>			<b>-58.73</b>	<b><i>cis</i>-FHU-779 Total</b>		<b>-53.40</b>		

Residues are designated by their genuine numbers in the rabbit Ca<sub>v</sub>1.2 channel and bracketed universal labels (see **Supplementary Table 1**). Residues, for which the absolute value of their energy contribution to the ligand-channel interaction energy is less than 0.5 kcal/mol are not shown, but their energy contributions are included in the total energies. Residues whose mutations are known to affect action of benzothiazepines (**Supplementary Table 1**) are underlined.



**Figure 1 | Design and characterization of the light-regulated diltiazem derivative FHU-779.**

(a,b) Chemical structures of (a) diltiazem and (b) its photoswitchable derivative FHU-779. Thermally stable *trans*-FHU-779 can be isomerized to its *cis*-state using 385 nm irradiation, while 470 nm irradiation or thermal relaxation ( $k_B T$ ) reverses the process. (c) The UV-Vis absorption spectra of FHU-779 under continuous irradiation at 380 nm, 480 nm or without additional illumination (dark). (d-g) Whole-cell patch clamp recordings of  $\text{Ca}_v1.2$   $\text{Ba}^{2+}$  currents in the presence of FHU-779 (25  $\mu\text{M}$ ) under 385 nm or 470 nm irradiation.  $\text{Ca}_v1.2$  channels were heterologously expressed in HEK293T cells together with the  $\beta_3$  and  $\alpha_2\delta_1$  auxiliary subunits. (d) I-V relationship of peak currents under 385 nm or 470 nm illumination. Voltage dependent currents were elicited by stepping from  $-70$  mV to  $+80$  mV in 10 mV increments at 1 Hz for 100 ms. Indicated color code applies also for (e) and (f). (e) A representative single current trace demonstrates reversible light-dependent block of  $\text{Ca}_v1.2$  by FHU-779 (25  $\mu\text{M}$ ). Displayed are the last triggered current-responses of 16 depolarizations under 385 nm and 470 nm irradiation, respectively. Currents were triggered by stepping from a holding potential of  $-70$  mV to  $+30$  mV at 1 Hz for 100 ms. Parallel lines represent time gap between currents. (f) Peak  $\text{Ca}_v1.2$   $\text{Ba}^+$  currents under 385 nm and 470 nm irradiation over multiple cycles. (g) Quantification of photoswitching induced by extracellularly or internally applied FHU-779 (25  $\mu\text{M}$  and 100  $\mu\text{M}$ , respectively). Statistical evaluation by one way ANOVA test,  $*p < 0.05$ ,  $**p < 0.01$ ,  $***p < 0.001$  (definition of photoswitching see online methods).

**Figure 2 | FHU-779 enables optical control of LTCC conductance.** (a,b) Ratiometric  $\text{Ca}^{2+}$  imaging of Fura-2- loaded HEK293T cells expressing  $\text{Ca}_v1.2\Delta 1671$ ,  $\beta_3$ ,  $\alpha_2\delta_1$  and TRAAK-GFP constructs in the presence of FHU-779. Cells were depolarized by  $\text{K}^+$  (60 mM) to activate LTCCs. (a) Representative  $\text{Ca}^{2+}$  signals in a single transfected HEK293T cell under alternating 380 nm and 480 nm illumination (40  $\mu\text{M}$  FHU-779). (b) Dose-response relationships of *trans*- and *cis*-FHU-779 determined by ratiometric  $\text{Ca}^{2+}$  imaging (see **Supplementary Fig. 3** and online methods). (c,d) The pore domain-model of the open L-type calcium channel with compound FHU-779 bound in the *trans*- (c) and *cis*- (d) conformations. The channel repeats I, II and IV are colored by yellow, magenta and cyan, respectively. For clarity, repeat III is not shown.

**Figure 3 | FHU-779 allows photoswitching of LTCC in pancreatic  $\beta$  cells, cardiomyocytes and mouse hearts.** (a) Reversible optical control of cytosolic  $\text{Ca}^{2+}$  levels measured by intensity-based  $\text{Ca}^{2+}$  imaging in mouse pancreatic islets using FHU-779 (35  $\mu\text{M}$ ). Representative  $\text{Ca}^{2+}$  signals from four islets (grey) imaged simultaneously ( $F/F_{\min}$  represents fluorescence/minimal fluorescence in respective trace). The black line represents the mean. (b) Whole-cell patch clamp experiments in dissociated  $\beta$  cells. Displayed as the I-V relationship in the presence of FHU-779 (35  $\mu\text{M}$ ,  $n = 7$  cells from 3 animals) under 370 nm and 470 nm irradiation. In all experiments, tolbutamide (200  $\mu\text{M}$ ) was added to block  $\text{K}_{\text{ATP}}$  channels and maintain LTCCs in the open state. (c) Insulin secretion under control conditions (11 mM glucose + 200  $\mu\text{M}$  tolbutamide) and following application of either diltiazem (50  $\mu\text{M}$ ), *trans*-FHU-779 or *cis*-FHU-779 (35  $\mu\text{M}$ ) ( $n = 4$  animals, circles represent individual data points). Statistical evaluation by one way ANOVA followed by Dunnett's multiple comparison test,  $*p < 0.05$ ,  $**p < 0.01$ . (d-f)  $\text{Ca}^{2+}$  imaging of FHU-779 block release in single  $\beta$  cells residing within intact pancreatic islets. (d) Representative image before and during targeted irradiation of ROI (1) with 405 nm light (photoactivated ROI, white solid line (1); control ROI, grey solid line (2); scale bar = 50  $\mu\text{m}$ ; representative images,  $n = 7$  islets from 3 animals). (e) Close up of photoactivated ROI (1) before and during 405 nm irradiation (colored map applies to d and e, W/O represents without). (f) Time course of  $F/F_{\min}$ , before and during block release of representative ROIs (1 and 2) ( $n = 7$  islets from 3 animals). (g,h) Heart rate modulation by FHU-779 (25  $\mu\text{M}$ ) in intact mouse hearts. (g) In the presence of FHU-779, 480 nm illumination of the right atrium decreased the spontaneous beating rate of a representative single heart. 380 nm reversed this effect. (h) Quantification of heart rate change induced by 380 nm light relative to 480 nm illumination before FHU-779 (W/O) and in the presence of FHU-779 ( $n = 4$  hearts, two tailed, paired student t-test,  $*p = 0.0375$ ). (i) Overlay of representative field potential (FP) signals of a monolayer Cor4U cardiomyocytes incubated with FHU-779 (10  $\mu\text{M}$ ). One electrode was in the illuminated region (i) of the MEA and the second electrode was in the non-illuminated area (Supplementary Fig. 9). The FP duration in (i) was determined to be  $170.4 \pm 14.8$ ,  $186.3 \pm 15.9$  ms for 480 nm and 385 nm, respectively ( $n = 4$ , paired t-test  $*p = 0.0159$ ).